Seed germination of four serotinous Agulhas Plain Proteaceae

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Seeds of *Protea susannae* Phill., *P. obtusifolia* Beuk ex Meisn., *Leucadendron coniferum* (L.) Meisn. and *L. meridianum* I. Williams were tested for their ability to germinate at different temperatures in the laboratory. Current year seed of all species had more than 90% germination at 10/20°C and 10/10°C. At 15/30°C germination was negligible (1 – 3%) in all species except *Leucadendron coniferum* where it was 44.4%. This could enable *L. coniferum* to germinate in response to warm season rains after spring burns. In all species canopy-stored seed of increasing age had similar high germination levels (>89%). Germination rates of older seeds were slower, however, than those of younger seeds, lagging between 2.5 to 6 days behind. This is interpreted as a bet-hedging strategy in response to dry spells after seed release.

Saad van *Protea susannae* Phill., *P. obtusifolia* Beuk ex Meisn., *Leucadendron coniferum* (L.) Meisn. en *L. meridianum* I. Williams is in die laboratorium getoets vir die vermoë om by verskillende temperature te ontkiem. Die huidige jaar se saad van alle spesies het meer as 90% ontkieming by 10/20°C en 10/10°C gelewer. Ontkieming by 15/30°C was gering (1 – 3%) vir alle spesies behalwe *Leucadendron coniferum* waar 44.4% ontkieming gevind is. Hierdie eienskap mag *L. coniferum* in staat stel om na reën gedurende die warm seisoen na 'n lente-vuur, te ontkiem. By alle spesies het saad wat in die kroon gehou word, met toenemende ouderdom dieselfde hoë ontkieming gelewer (>89%). Die tempo van ontkieming van ouer sade was stadiger as dié van jonger sade, met 'n agterstand van 2.5 tot 6 dae. Dit kan geïnterpreteer word as 'n strategie wat ontkieming vertraag gedurende wisselvallige omstandighede tydens droë periodes na die vrystelling van saad.

Keywords: Bet-hedging, fynbos, germination, Proteaceae, serotiny.

Introduction

Seedling emergence in fynbos is largely confined to the first winter after fire. Laboratory studies on the germination requirements of Proteaceae seeds have included the effect of inhibitors, promotors, oxygen, hormones and scarification treatments (van Staden 1966; Brown & van Staden 1971, 1973, 1975; van Staden & Brown 1977; Brown & Dix 1985). The above studies were not carried out in an ecological context (e.g. as part of a syndrome of myrmecochory or serotiny) in that the results did not aim to describe processes which explain behaviour in the field.

Recently, laboratory trials have shown a close relationship between the oxygen and temperature requirements of the nut-like seeds of myrmecochorous Proteaceae, and their environments (Brits 1986a, 1986b; Brits 1987). Brits (1986b) showed that the daily temperature fluctuations required for germination of seeds of the myrmecochorous species *Leucospermum cordifolium* (9°C/24°C) and *Serruria florida* (7°C/20°C) closely resembled the post-fire soil temperatures in their respective habitats. The importance of low temperatures for the successful germination of seeds of serotinous species has been suggested by Bond (1984). Corroborative evidence shows that in the field germination occurs in the cold winter months (Bond 1984; Midgley 1989). Deall and Brown (1981) found that low-temperature (5°C) incubation significantly improved the germination of *Protea magnifica* seeds, and Brown and van Staden (1971) found that in three out of five Proteaceae species, higher levels of germination occurred at 15/20°C than at 20/30°C.

Since much ecological importance is attached to the serotinous habit (Lamont *et al.* 1991), it is important to know the levels of viability of different ages of canopy-stored seeds. Bond (1985) and Cowling *et al.* (1986) found varying patterns of viability with increasing age of canopy-stored seed for South African and Australian Proteaceae respectively. Le Maitre (1990) found that although germination success of canopy-stored seed did not decline with age, germination rates did.

In this study we performed laboratory tests to investigate germination characteristics of four Agulhas Plain Proteaceae: *Protea susannae* Phill., *P. obtusifolia* Beuk ex Meisn., *Leucadendron coniferum* (L.) Meisn. and *L. meridianum* I. Williams. We tested whether low temperatures alone, or daily fluctuating temperatures, are necessary for germination by incubating seed at 10/20°C, 10/10°C and 15/30°C. We also investigated the viability and germination rates of different ages of canopy-stored seed. The results are interpreted ecologically.

Study site and materials

The study species are all non-sprouting, serotinous proteoid shrubs. Their achene-type fruits are commonly referred to as 'seed'. The species occur as dominants in Proteoid fynbos of the coastal lowlands of the Agulhas Plain (Cowling *et al.* 1988). *Protea obtusifolia* and *Leucadendron meridianum* co-occur on the soils overlying limestone, and *P. susannae* and *L. coniferum* co-occur on the adjacent colluvial sands. Seed used in this study was collected from 30 plants per species in 18-year-old populations growing in the Heuningrug area, south of Bredasdorp (34°35′ S, 19°55′ E).

Methods

Seed of different ages was obtained from cones collected during March 1987. Cones aged 0 – 1 (current), 2, 3, 4

years and older for *Protea* spp. and 0 – 1, 2, 3, 4 and 5 years and older for *Leucadendron* spp. were oven-dried at 40°C for 48 h to promote seed release. The apparently viable (plump and heavy), aborted and predated seed were sorted by hand. In order to assess germination at different temperature regimes, one hundred apparently viable current seeds (10 seeds in 10 Petri dishes) were incubated on filter paper with 3 ml water containing 0.075% Benlate (fungicide), simulating optimal moisture conditions. Moisture levels were maintained by the addition of water. The use of fungicide was recommended by Benic and Knox-Davis (1983). Petri dishes were placed in controlled environment growth cabinets under the following alternating temperature ranges: 10/20°C, 10/10°C and 15/30°C, all under alternating dark/light conditions for 14/10 h. Germination of differently aged seed was determined at 10/20°C. Germination was taken as the emergence of the radicle. In order to investigate the relationship between germination rate and seed age, the number of germinated seeds was noted at intervals of about four days. After 40 days, ungerminated seeds were cut open to establish the presence of an embryo. Germination success was calculated as the total number of seeds germinated as a percentage of apparently viable seeds that had full embryos.

One-way analysis of variance was performed on the arcsine of the square root transformations (Zar 1984) of the percentage germination data. Two-way analysis of variance was used to determine the effect of increasing age of canopy-stored seed of each of the four species, on the time taken to germinate. Statgraphics software (Statistical Graphics Corporation) was used for these analyses. The Kolmogorov–Smirnoff two-sample test (Siegel 1956) was used to determine differences in the cumulative germination distributions over time, of seed of increasing age.

Results

Current seed of all four species had equally high (>90%) germination success at both the 10/20°C and 10/10°C temperature regimes (Table 1). At the higher temperatures of 15/30°C, germination was negligible in all species except for *Leucadendron coniferum* where 44.4% of the seed germinated.

Within each species, canopy-stored seed of all age categories also had equally high (>90%) germination, with the exception of 4-year-old seed of *Protea obtusifolia* which had a significantly lower germination (89%) than the younger age categories (Table 2). Despite these similarities in final germination percentages, there were differences between age categories in cumulative germination distributions over time, with older seed generally lagging behind younger seed by 2.5 to 6 days. Current seed germination of *P. susannae* had a significantly different distribution in time from its 4-year-old seed (Table 3). Germination rates of current seed of *P. obtusifolia* differed significantly from those of older seed categories, and *Leucadendron coniferum* current seed differed from its 3-, 4- and 5-year-old seed. There were also different distributions between 2-, and both 4- and 5-year-old seed, as well as between 4- and 5-year-old seed in *L. meridianum*. In all these instances, seed within older age categories took longer to germinate than the younger seed. This is reflected in the time taken for fifty percent of seeds to germinate (Tables 4a and 4b). There were highly significant differences between age categories of the combined species, with the 3- and 4-year-old seed taking longer than the younger current and 2-year-old seed. Both *Leucadendron* spp. had significantly faster germination times than the *Protea* spp. The interaction between age classes and species was also highly significant.

Table 1 Percentage germination of the current year's seed of *Protea susannae, P. obtusifolia, Leucadendron coniferum* and *L. meridianum* at different temperature regimes[x]

	10/20°C	10/10°C	15/30°C
P. susannae	93.9 (3.1)	95.0 (3.1)^{ab}	1.0 (1.0)^{a}
P. obtusifolia	99.0 (1.0)	98.0 (1.3)^{ab}	3.0 (1.5)^{a}
L. coniferum	96.0 (1.6)	99.0 (1.0)^{b}	44.4 (5.0)^{b}
L. meridianum	92.6 (2.6)	90.6 (2.8)^{a}	2.0 (1.3)^{a}
	NS	*	***

[x] Data are mean (SE) percentage seed germinating per Petri dish. Significant differences between species at each temperature regime were determined by one-way analysis of variance. Data were transformed to the arcsine of the square root. Different letters (a, b, c, d) indicate significant differences according to Tukeys multiple-range test. n = 10 Petri dishes. NS: not significant; *: $P < 0.05$; ***: $P < 0.001$.

Table 2 Percentage germination of canopy-stored seed of increasing age of *Protea susannae, P. obtusifolia, Leucadendron coniferum* and *L. meridianum*[x]

Time stored on plant (years)	*P. susannae*	*P. obtusifolia*	*L. coniferum*	*L. meridianum*
0 – 1	93.9 (3.1)	99.0 (1.0)^{a}	96.0 (1.6)	92.8 (2.6)
2	97.0 (1.5)	99.0 (1.0)^{a}	98.0 (1.3)	95.0 (2.2)
3	99.0 (1.0)	99.0 (1.0)^{a}	93.9 (2.2)	91.3 (4.1)
4	97.0 (1.5)	89.0 (2.8)^{b}	96.0 (1.6)	94.4 (2.4)
>4	—	—	96.0 (2.2)	95.9 (1.7)
	NS	***	NS	NS

[x] Data are mean (SE) percentage seeds germinating per Petri dish. For each species significant differences between years were determined by one-way analysis of variance. Data were transformed to the arcsine of the square root. Different letters (a, b) indicate significant differences according to Tukeys multiple-range test. n = 10 Petri dishes. NS: not significant; ***: $P < 0.001$.

Table 3 Differences in cumulative germination distributions over time of canopy-stored seed of different ages of *Protea susannae, P. obtusifolia, Leucadendron coniferum* and *L. meridianum*[x]

Protea susannae	*Protea obtusifolia*	*Leucadendron coniferum*	*Leucadendron meridianum*
Yr 1 : yr 4***	Yr 1 : yr 2***	Yr 1 : yr 3***	Yr 2 : yr 3***
	Yr 1 : yr 3***	Yr 1 : yr 4***	Yr 2 : yr 5***
	Yr 1 : yr 4***	Yr 1 : yr 5***	Yr 4 : yr 5***

[x] n = 10 Petri dishes. The Kolmogorov–Smirnoff test was used to test for differences between distributions. Only significant relationships are given. ***: $P < 0.001$.

Discussion

Unlike the daily fluctuating temperatures needed for the germination of two myrmecochorous Proteaceae (Brits 1986a), all four species in this study had equally high

Table 4a Germination rates of increasing ages of canopy-stored seed of *Protea susannae*, *P. obtusifolia*, *Leucadendron coniferum* and *L. meridianum*[x]

Time stored on plant (years)	*Protea susannae*[b]	*Protea obtusifolia*[c]	*Leucadendron coniferum*[a]	*Leucadendron meridianum*[a]
0 – 1[a]	20.9 (0.5)	25.1 (0.2)	16.9 (0.4)	18.1 (0.5)
2[a]	20.1 (0.4)	29.3 (0.7)	16.9 (0.3)	16.8 (0.5)
3[b]	20.9 (0.6)	30.3 (0.5)	19.5 (0.5)	19.4 (0.5)
4[b]	23.5 (0.3)	31.1 (0.7)	18.7 (0.3)	16.3 (0.3)
>4	—	—	19.9 (0.4)	19.2 (0.4)

[x] Data are times (days) taken for fifty percent of a test sample to germinate. n = 10 petri dishes. The effect of seed age and species on germination rates was determined by two-way analysis of variance. Different letters (a, b, c) indicate significant differences between increasing ages of canopy-stored seed for combined species, and between species for combined years, according to Tukeys multiple-range test.

Table 4b Two-way analysis of variance on the effects of age (0 – 4 years) of canopy-stored seed, and species, on the time taken for fifty percent of seed to germinate

Source of variation	dF	F	P
Age of canopy-stored seed	3	24.2	<0.001
Species	3	504.3	<0.001
Age of canopy-stored seed × species	9	11.8	<0.001

germination at a constant level of 10°C, as when incubated at temperatures fluctuating between 10 and 20°C. This fits the suggestion that, as a drought-avoiding mechanism, low temperatures are required for serotinous Proteaceae to germinate (Bond 1984). Since seeds of serotinous species are not stored in the soil, there is no selection for the recognition of a specific post-fire signal (fluctuating temperatures) as is the case for myrmecochorous species (Brits 1986a). In a separate study (P.J. Mustart & R.M. Cowling, unpublished data), field-monitoring of the post-fire environments in which seed of these study species were planted, showed that seedling emergence occurred during a period when soil surface daily minimum temperatures ranged between 7 and 10°C (June to August), suggesting good agreement between laboratory and field germination trials.

Almost half the seeds of *Leucadendron coniferum* had less strict dormancy-breaking requirements and germinated at a higher temperature regime (15/30°C). If we assume that it is the minimum (15°C) temperatures that are controlling germination, then it can be predicted that this species will be able to germinate in the field over longer periods than the other species in this study, providing sufficient moisture is available. Minimum soil temperatures are rarely higher than 15°C throughout the whole year, though maximum temperatures are often higher than 30°C from late spring to early autumn (P.J. Mustart & R.M. Cowling, unpublished data). We predict that this species would be better able to survive after spring burns than the others, since some germination and seedling establishment could occur during the relatively wet spring months. Seeds would thus not be exposed to both heat (Cowling *et al.* 1986) and predation (Bond 1984) over the summer months. The existence of pure populations of this species in certain areas of the Agulhas Plain, when coexistence with *Protea susannae* is more common, could be explained by a history of spring burning. It would be necessary to monitor seedling emergence after a spring burn to confirm this.

It can be problematic to extrapolate the significance of laboratory findings to germination behaviour in the field. However, of interest was that not only were there differences in germination requirements between species, but there were also differences in germination behaviour within species. The latter was evident in the seed age-specific germination rates, with older seeds generally taking between 2.5 to 6 days longer to germinate. This time lag would enable the older seeds in the seed bank to remain in the early passive imbibition stages, prior to the later drought-sensitive growth stages (Hillel 1972). These 'slower' seeds would be immune to subsequent desiccation. Variable seed germination (seed polymorphisms, or heterocarpy) has been described as bet-hedging strategy in variable and unpredictable environments (Cohen 1968; Harper 1977). The need for bet-hedging in arid areas with sporadic rain is easily understood, and is well documented for many species (Mayer & Poljakoff-Mayber, 1975). There might appear to be little need of a bet-hedging strategy for Proteaceae species growing in areas with reliable winter rainfall. However, fynbos Proteaceae that are killed by fire are vulnerable to germination setbacks since they rely totally on their seed for re-establishment of post-fire populations. Since seed lie on, or near to, the soil surface, any temperature-, wind- or drainage-induced micro-habitat changes in moisture would have important consequences for the imbibing seed. A spread of germination in time, acting as a bet-hedge against this, would optimize germination. Zammit and Westoby (1987) found that seeds of the obligate reseeder *Banksia ericifolia* germinated over twice the time than that taken by seeds of the resprouter *B. oblongifolia*, and they suggested that this is a risk-spreading mechanism to reduce subsequent seedling mortality in areas where rainfall is unpredictable. Since it is the older seed of our species that has the longer germination times, we propose that the strategy of retention of these older seeds on the canopy in Proteaceae is aimed at maximizing post-fire germination in drier, more variable environments where little or no inter-fire recruitment occurs. Both Bond (1985) and Cowling and Lamont (1985) have observed a higher degree of serotiny in xeric than in mesic areas for fynbos and south-western Australian Proteaceae, respectively.

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